Principal Investigator/Program Director (Last, first and middle):

INCLUSION OF CHILDREN

Inclusion of Children. Children will not be included in the study.

VERTEBRATE ANIMALS:

1. Detailed description of animal use.
All work with vertebrate animals will be conducted in China.
Capture and sampling techniques for all [illegible] UC Davis IACUC (Mazet and Epstein, UC Davis 15898, current).

Experiments [illegible] lab of Wuhan University at the School of Medicine in Wuhan, China. The Center is AAALAC accredited and has both an Institutional Biosafety Committee and an Institutional Animal Care and Use Committee. Animals will be housed in a BSL-2 facility and will be under the care of a full-time veterinarian. We [illegible] protocols for IACUC approval should this proposal be funded. Conditions for animal use are described below.

Note: The majority of wild animals captured and sampled will be done using non-destructive techniques [illegible] small number of instances [illegible] required to establish [illegible] according to accepted protocols (see euthanasia section).

Bat capture. Free-ranging bats will be captured [illegible] mist net or harp trap. Traps will be manned by two people during the entire capture period, and bats are removed from the nets as soon as they become entangled to minimize stress and prevent injury. In the Co-PI's (Dr. Epstein) experience, a maximum of 25-30 bats can be safely held and processed by a team of three people per trapping period. Duration of trapping will depend on the capture rate. Bats are placed into a pillowcase or small cloth bag and hung from a branch or post until samples are collected. Bats are held for a maximum of [illegible] hours.

Wild rodent capture. Free-ranging rodents will be captured through [illegible] including resident free-ranging [illegible] traps. Traps will be checked [illegible] expected or researchers are working in areas where predation is common, traps will be checked frequently, and closed during the adverse weather. Handling procedures will involve morphometric measurements. Captive and wild rodent sampling procedures (including euthanasia if necessary), will involve manual restraint, venipuncture, mucosal [illegible] collection. Following capture, animals will be restrained with a [illegible] ensure the animals are not traumatized by the hoop of the net or through net removal. Larger rodents will be restrained for sampling in specialized squeeze cages, allowing adjustments appropriate to the size of the animal. Squeeze-cages consist of [illegible] to press the animal, while ensuring visible communication between the field veterinarian and the animal. Once squeezed, a rod is inserted to keep the plastic shield in place. The box is then inverted, allowing sampling to be conducted through the open wire bottom and abdomen of the animal when the animal is safely immobilized. Anesthesia for small rodents will be conducted using plastic tubes, with the animals transferred directly from the traps to [illegible] induction. For larger rodents, chemical restraint and anesthesia (ketamine alone, or ketamine combined with xylazine) will be applied either through the squeeze cages by syringe if applicable.

Laboratory animals. Laboratory mice will be sourced commercially by the Wuhan Center for Animal Experiment at Wuhan University.

Sample Collection. Bats will be manually restrained during sampling.
Bats: Depending on the species and size of bat, swabs will be taken from the oropharynx, urogenital tract, and rectum. Fresh fecal pellets will be collected if available, in which case a rectal swab will not be collected. Blood will be collected from fruit bats either from the cephalic vein or from the radial artery or vein using a 25 gauge needle and 1cc syringe. Blood will be collected from bats weighing less than 100g according to published techniques (726).

Rodents: Rodents will be anesthetized prior to sampling.
Once anesthetized a small blood sample will be collected using a capillary tube placed into the retro-orbital sinus. Ma
rodents. Femoral or jugular venipuncture may be used for larger rodents (e.g. rats). In all
volumes of no more than 1% of body weight will be withdrawn (example 0.2 ml blood from a 20 gram rodent).

Civets and other small carnivores: Ketamine will be used to restrain small free ranging mammals according to published protocols. Animals will be monitored continuously while recovering from anesthesia. Animals that are sampled in the marketplace, and that may potentially be consumed, will not be anesthetized. Manual restraint will be used and blood will be drawn from the femoral artery or jugular vein.

Laboratory Mice: Humanized mice will be bred at the University of [redacted]. Mice will be challenged with a specific dose (e.g. 1x10^6 TCID50) of virus through different routes (intranasally and intraperitoneal). Mouse body temperature will be monitored using implantable temperature transponders (Destron Fearing), and mice will be weighed daily. Animals will be observed daily for clinical signs of disease. Moribund mice will be euthanized, according to AVMA recommendations. Live animals will be euthanized at three weeks post-challenge. Blood will be collected to measure neutralizing antibodies against bat CoVs. We will collect nasal washes, oral swabs, rectal swabs, and urine every two days. These are minimally invasive procedures, and will be performed by experienced lab technicians under the supervision of a full veterinarian.

2. Justify use of animals: The choice of species, numbers to be used, species and number used in study.
The purpose of this study is to identify novel coronaviruses that may pose a risk to the health of both humans and animals. The experimental work is designed to understand the ability of bat coronaviruses to bind to human receptors. Because we don't have prevalence estimates for [redacted], we have used a conservative estimate of 10% prevalence. SARS-like coronaviruses have been found in between 10% and 38% of bats studied (4, 20). A 10% in wild populations of bats would require a sample of 30 individuals per species for detection of an infected individual with 95% confidence. **Wild bats:** We will sample 30 individuals from 30 different species in each province in China (2 per species euthanized). Species will be limited to the following families: *Rhinolophidae, Hipposideridae, Vespertilionidae, Miniopteridae, Molossidae, Emballonuridae, Pteropodidae*, all of which are present in Southern China and potentially in the wildlife markets. **Bats in wet markets:** We will opportunistically sample a wide variety of insectivorous and frugivorous bats that is present in markets. In addition to bats, we will sample civets, non-human primates, rats, and other rodents present in the markets that may act as intermediate hosts. Numbers sampled from markets will be limited to animal availability. However, [redacted] sampling of wildlife will be conducted in the most humane manner while minimizing the impacts on individual animals and their wild populations. In cases where feces are collected for testing, non-invasive techniques will be used. In all instances, the fewest number of animals will be sampled that will provide valid information and statistical inference for the pathogen and disease of interest and every effort will be made to minimize stress or discomfort for the animal.

A small number of bats (maximum 2 per species) representing each of the species in this study may be euthanized in order to collect lung and gut intestinal tissue, which is required for characterizing coronavirus nature. Voucher specimens may also be collected at the discretion of the team leader for the accurate identification of species using molecular methodology.

Humanized mice for experimental infection: For Specific Aim 3, in order to test the ability of bat coronaviruses that utilize receptors found in people have the potential to infect people, we will use SARS-CoV binding mice (standard breed at Wuhan University) that have been genetically modified to express human receptors. We'll infect these with cultured bat coronaviruses and determine which organs become infected and whether these mice are capable of shedding infectious virus. Humanized mice will be genetically modified to carry human ACE2 or DPP4 gene. We will be used to evaluate the pathogenesis of CoVs. We cannot anticipate exactly how many viruses we will find that are candidates for experimental models, however we estimate that we will use

four adult mice (2 male, 2 fem[illegible] [illegible]
for mouse infection experiments. [illegible] mice over the study p[illegible].

3. Pr[illegible] on veterinary care: [illegible] there is no specific veterinary care th[illegible]
is appropriate, no[illegible] clinical veterinary facilities be available. Animals that ar[illegible]
sampling process will be assessed by an experienced team [illegible]ader and if the animal is [illegible]ined to be
unlikely to survive if released, it shall be euthanized humanely (see euthanasia section). Animals will be
rele[illegible] or anesthesia.
Animals will be returned to vendors after sampling [illegible]
released in the area outside the marketplace.

Laboratory mic[illegible] used in the [illegible]
University. Experimental animals w[illegible] regularly monitored by experienced staff and a supervising
veterinarian. The animal facility [illegible] 24 hours a [illegible]
will be provided [illegible]

4. Pro[illegible]cedures for ens[illegible]ring animal comfort, lack of distres[illegible] pain, or injury: Animals will not be held
longer than 6 hours. Co-PIs, Drs. Epstein and Olival have extensive experience in c[illegible]
sampling wildlife, including bats. In our experience, bats and rodents tolerate the described procedure well.
M[illegible]ets will be attended cont[illegible]uously during capture periods, and bats will be extracted from the net as soon
as they become entangled. This will [illegible]
individually in cotton bags and hung from tree branches while awaiting processing and during recovery. The
bags are suf[illegible]
environ[illegible]s to c[illegible] the bats [illegible], but they hang quietly. [illegible]
be monit[illegible] of capture, processing [illegible]
release. An[illegible]mals [illegible]
traps will be checked in the morning while it still cool outside. Rodents will be kept in a cool, shaded
en[illegible] within 10 hours of capture.

The procedures used in this experiment (blood [illegible]raw, nasal, ora[illegible], and [illegible]
however, mic[illegible] that s[illegible]g[illegible]ns of morbidity post-infection will be examined and [illegible]hanized acco[illegible] AVM[illegible]
standards (see below).

5. Euthanasia: In the event of inju[illegible] an animal that results in [illegible]
care is unavailable, [illegible] animal will be e[illegible] by a veterinarian or trained field team member [illegible]
ketamine injected intramuscularly [illegible]5mg/kg and sodium pentobarbital injected intravenously at a dose of
1.0ml per 5kg injected intravenously. This protocol is in accordance with the AVMA euthanasia report [illegible]
Any animal that is euthanized usi[illegible] [illegible]
the food supply either through markets or hunting.

S

SARS-CoV caused outbreaks with significant case fatality rates, and there are no vaccines available for this agent. S[illegible] classified as a BSL-3 agent. The work proposed in this application will involve two aspects: [illegible] CoVs, while working in caves with high bat density overhead and the potential for fecal dust to be inhaled. There is also s[illegible] risk of exposure to pathogens or physical injury while handling bats, rodents or other animals, their blood samples or their excreta. The Co-PI is a veterinarian with extensive experience working with wildlife species and high-biosecurity pathogens (N[illegible] taken in the field to limit the risk [illegible] strict procedures for handling bats and working with samples from [illegible] as they are secured in the field and transported to the lab. Field team members handling animals will be trained to utilize personal protective equipment and practice proper environmental disi[illegible] techniques. This includes wearing coveralls, dedicated clothing, nitrile gloves, eye protection, and a P95 or P100 respirator. All field clothing and equipment will be disinfected using Virkon disinfectant. All biological waste from field surveys will be disposed of in the appropriate [illegible] labs. All personnel will be vaccinated against rabies [illegible] WHO and CDC recommendations. Field teams will carry rabies boosters in the field and will administer a booster in the event of a potential rabies exposure.

Field safety protocol: [illegible] washed roughly with soap and water to clean [illegible] gauze bandage and benzalkonium chloride for 5 minutes. If bleeding, pressure is applied with a sterile bandage for until bleeding has stopped. If the wound continues to bleed, medical attention at the nearest hospital is sought. The bat from which the bite or exposure originated is identified, and the samples collected from it labeled on the data sheet that these were involved in an exposure. Our procedures require that the person potentially exposed [illegible] a major hospital within 24 hours [illegible] rabies booster (as per WHO/CDC [illegible] Samples [illegible] buffer will be non-infectious. Samples placed in viral trans[illegible] temperatures (-86C) until viral isolation is required. Serum will be heat inactivated (56C [illegible] prior to testing.

Lab biosafety: Wuhan Institute of Virology and the Wuhan University Center for Animal Experiment BSL-3 lab have an internal Biosafety Committee and are accredited BSL-2 and BSL-3 laboratories. All experimental work using infectious material will be conducted under [illegible] Disposal of hazardous materials will be conducted according to [illegible]

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CONSORTIUM/CONTRACTUAL ARRANGEMENTS

Consortium/Contractual Arrangements

This project is a multi-institutional collaboration led by EcoHealth Alliance, New York (Daszak, PI), which will subcontract direct funds to two institutions: the East China Normal University (Dr S. Zhang) and the Wuhan Institute of Virology (Dr. Z. Shi), which are both foreign institutions. Dr. Daszak has over 15 years experience managing collaborative projects in China, including a 5-year NSF/NIH Ecology of Infectious Diseases award on West Nile virus with one of the subcontractees, an R01 on bat viral discovery that involves multiple international contracts, a $1 million dollar p.a. contract from the DoD (which EcoHealth Alliance) is justified in taking the lead on this and virology processes underlying zoonotic disease emergence). preliminary work on this issue including 10 years of research on the ecology of SARS and the emergence of SARS and 11-years of work in China. The subcontractees will work on special issues and areas in which they have proven expertise. These areas are: human and animal [illegible] sampling (East China Normal University, Dr. Zhang) and viral discovery, pathogenesis as well as sample storage and characterization (Wuhan Institute of Virology, Dr. Shi). Dr Daszak has [illegible] and co-directed a joint institute in China with Dr. Zhang, and has been involved in contractual arrangements with ECNU for 8 years. Dr. Shi Zhengli and Dr. Daszak collaborated together since 2002 and have been involved in running joint conferences, and shipping samples into and out of China.

RESOURCE SHARING PLAN:

Data Sharing Plan: [illegible] sequence data will be made publicly available via GenBank, and by other scientists, as soon as a publication is in press. Viral isolates will remain at the Wadsworth Center Virology initially. Isolates, reagents and any other products, should they be developed, will be available to [illegible] Transfer Agreements and/or licensing agreements.

Sharing Model Organisms: We do not anticipate that any [illegible] Should any be developed, they will be made available [illegible] Institute of Virology and EcoHealth Alliance Material Transfer Agreements and/or licensing agreements.

Genome Wide Association Studies (GWAS): N/A

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The res[illegible] ued [illegible] e R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Fede[illegible]l Identifier: GRANT11418218

2. Change o[illegible]nvestigator / Change of Institution Questions

☐ Change of pri[illegible]pal inve[illegible]

Name of former principal investigator / program director:

Prefix:

[illegible]st Name:

Middle Name:

* Last Name:

Suffix:

☐ Cha[illegible] of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For rene[illegible]

* Invention[illegible]

[illegible]answer is Yes[illegible]

* Previously Repo[illegible] Yes ☐ No ☐

4. * Program Income

Is program income anticipated during the period(s) for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" [illegible] amount and

5. * Disclosure Permission Statement

If this application does not result in an award, is it permissible for the Government to disclose the title of your application and your name to persons interested in contacting you for further information (e.g., potential collaborators)?

☒ Yes ☐ No